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In Vivo Toxicity and In Silico Molecular Docking of NXH8, a Post-Synaptic Three-Finger Toxin from *Micrurus corallinus*, in Comparison to α-Bungarotoxin

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INTRODUCTION & AIM

Micrurus corallinus is a coral snake endemic to Brazil, whose venom is known to contain toxins with both pre- and postsynaptic activity. Recently, we observed that NXH8, a three-finger toxin (3FTx) from *M. corallinus*, exhibited antagonistic action on the nicotinic acetylcholine receptor (nAChR) of *Tetronarce californica* in phrenic nerve-diaphragm preparations from mice. However, this action was found to be easily reversible, suggesting a potentially low toxicity. In this study, the in vivo toxicity of NXH8 was evaluated, alongside *in silico* analyses comparing the interactions of NXH8 and α-bungarotoxin (α-BgTx) from *Bungarus multicinctus* with the nAChR of *T. californica*.

METHOD

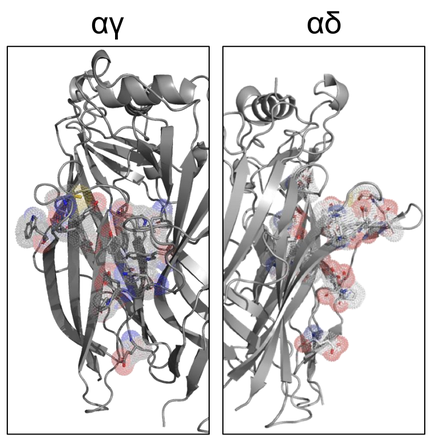
Evaluation of Anti-NXH8 Antibodies and Survival Assays in Mice

Anti-NXH8 antibodies were produced in Balb/c mice. *In vivo* toxicity was assessed in mice receiving 3 LD50 of *M. corallinus* venom (control) or synthetic NXH8. The neutralizing capacity of anti-NXH8 antibodies was tested in mice injected with venom pre-incubated with saline (G1), antivenom (G2), anti-NXH8 antibodies (G3), and post-treated with Varespladib (VPL) intramuscularly after intraperitoneal administration of venom pre-incubated with saline (G4) or pre-incubated with anti-NXH8 antibodies (G5). Survival was monitored for 48 hours.

Comparative In Silico Analysis of NXH8 and α-BgTx Interactions with nAChR

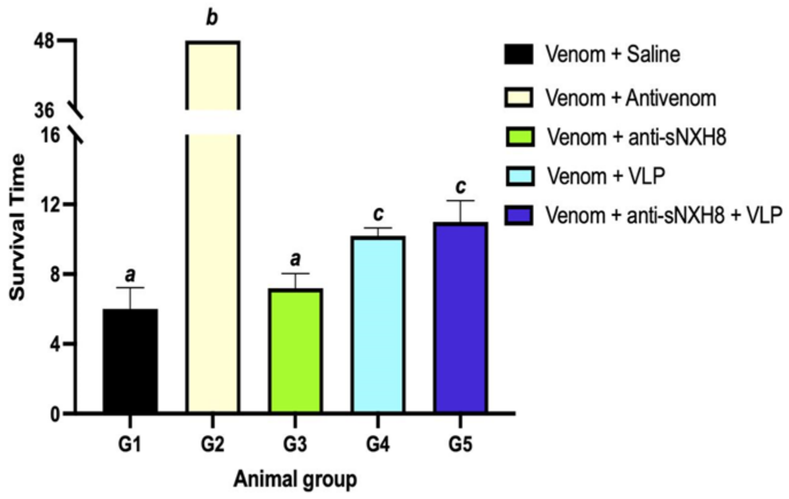
In silico analyses were conducted to understand the molecular interactions between NXH8 and the nAChR of *T. californica*, comparing them to the interactions of α-BgTx, with high affinity for the same receptor. NXH8 docking with the *T. californica* nAChR was performed using HADDOCK 2.4, with structures predicted by AlphaFold2. Residues at the αγ and αδ interfaces were identified from predicted contacts: active residues were manually selected, and passive residues were automatically assigned by HADDOCK based on proximity to active sites.

Interface	Chain	Active residues	Passive residues
αδ	α	D93 W149 W187 Y189 Y190 T191 C193 Y198	D89 L90 N94 A96 Y127 E129 K145 G153 S157 S159 K185 H186 V188 C192 P194 D195 T196 P197 L199
	δ	T38 W57 L111 D180 P181 E182 E186	D59 I79 Y106 N109 R113 Y117 T119 L121 I178 I179 A183 F184 T185 G188 E189 E191 K224
αγ	α	Y93 W149 W187 Y190 C193 Y198	D89 L90 N94 A96 E129 K145 G153 S157 S159 K185 H186 V188 Y189 T191 C192 P194 D195 T196 P197 L199
	γ	W55 L119 H172 D174 P175 E180	K34 T36 Y104 N107 L109 Y117 E163 W170 I173 E176 D177 F178 T179 G182 E183 T185 R189 K218



Active and passive residues at the αγ and αδ interfaces of the T. californica nAChR.

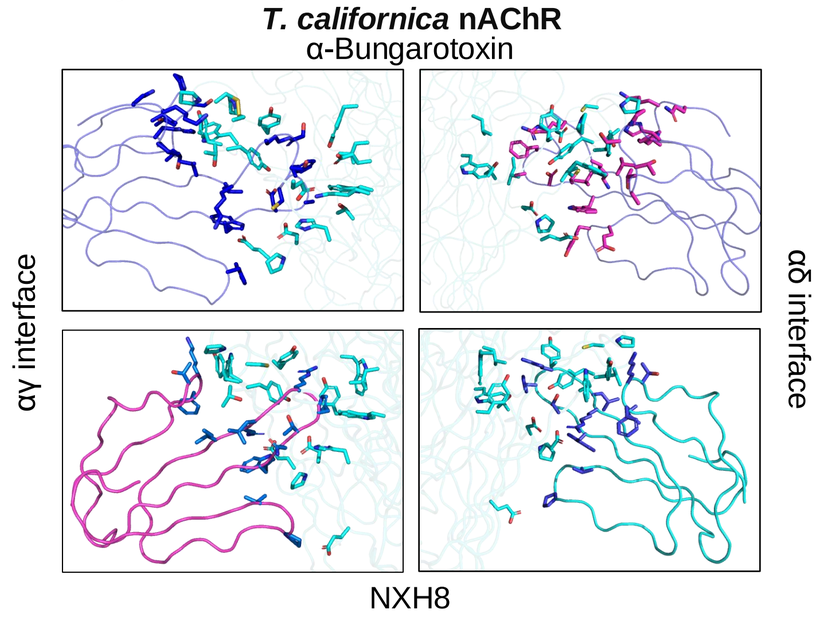
RESULTS & DISCUSSION



Survival assay with anti-sNXH8 and VLP

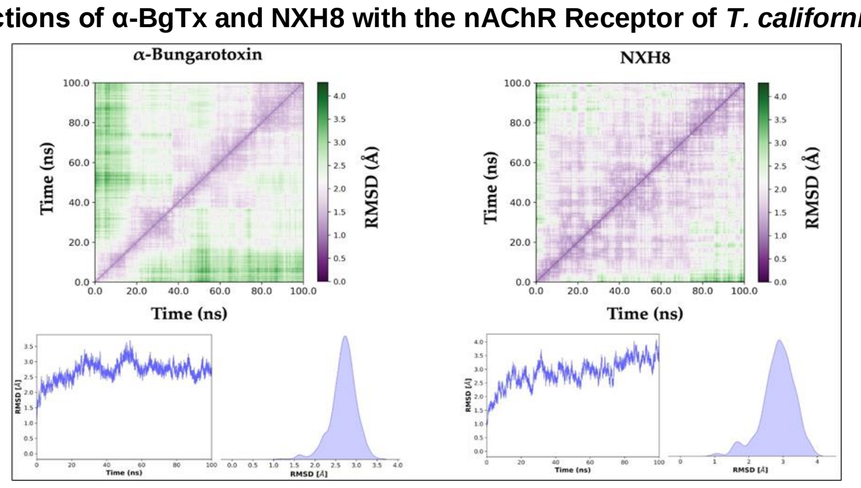
Evaluation of the ability of anti-sNXH8 antibodies and VLP to neutralize the toxicity of *M. corallinus* venom. Different letters indicate statistically significant differences between groups ($p < 0.05$). Identical letters denote no significant difference between groups ($p > 0.05$).

Comparative Analysis of α-BgTx and NXH8 Interactions at the αδ Interface of *T. californica* nAChR



At the αδ interface of the nAChR, a comparison of residues from Finger II of α-BgTx and NXH8 highlights key differences in their interactions. In α-BgTx, residues such as Arg36, Asp30, and Lys38 from Finger II establish a robust network of interactions. Specifically, Arg36 interacts with αThr191, αCys192, and αTyr198 on the α subunit, while also engaging δAsp180 and δTrp176 on the δ subunit. These interactions are supported by Asp30 and Lys38, which contribute additional stabilising electrostatic and hydrogen bonds. In contrast, Finger II of NXH8 forms a more restricted set of interactions. Key residues such as Arg35 and Arg38 interact with αTyr190, αThr191, and αCys192 on the α subunit, as well as δThr38 and δTrp176 on the δ subunit. However, the absence of dense aromatic and electrostatic interactions, as seen in α-BgTx, limits the overall stability of the binding network. While Arg35 and Arg38 provide some degree of interaction strength, their contribution is less extensive compared to the robust network formed by the corresponding residues in α-BgTx.

Comparative Analysis of 2D Maps, Temporal Graphs, and RMSD Histograms for the Interactions of α-BgTx and NXH8 with the nAChR Receptor of *T. californica*.



2D RMSD maps, time plots, and histograms for *T. californica* nAChR with BgTx (left) and NXH8 (right). BgTx shows broader conformational variation (green regions, higher histogram peak), indicating instability, while NXH8 displays lower variation (purple regions, stable RMSD), suggesting a stabilising interaction.

CONCLUSION

NXH8's low toxicity in vivo is likely due to structural differences from α-bungarotoxin, suggesting that other venom toxins, including presynaptic β-neurotoxins such as Phospholipase A_2 (PLA_2), may contribute to lethality. This study was conducted under Animal Ethics Committee Protocol 4463100419 with financial support from H.R-R. (FAPESP: 2017/18398-1) and S.H. (CNPq: 406816/2022-0).

FUTURE WORK / REFERENCES

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[4] Roman-Ramos H, Ho PL. Current Technologies in Snake Venom Analysis and Applications. Toxins (Basel). 2024 Oct 25;16(11):458. doi: 10.3390/toxins16110458. PMID: 39591213; PMCID: PMC11598588.